I. The Rejection of Claims 130-133, 136-145, 151-155, and 158-168 under 35 U.S.C. § 112, First Paragraph

Claims 130-133, 136-145, 151-155, and 158-168 stand rejected under 35 U.S.C. § 112, first paragraph, "as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. The Office Action states:

> The specification discloses SEQ ID NO's: 1 and 2 which correspond respectively to the nucleotide sequence and the amino acid sequence of an Aspergillus aminopeptidase. These SEQ ID NO's meet the written description provisions of 35 U.S.C. 112, first paragraph. However, the claims are directed to or encompass corresponding polypeptides from other species, mutated peptides, peptides produced from allelic variants, splice variants, peptide sequences that have a recited degree of identity and peptides which are encoded by hybridizing nucleic acids. None of these sequences meets the written description provision of 35 U.S.C. 112, first paragraph.

This rejection is respectfully traversed.

The present invention is directed to polypeptides having aminopeptidase activity with physicochemical properties of (i) a pH optimum in the range of from about pH 7.27 to about pH 10.95 determined at ambient temperature in the presence of Ala-para-nitroanilide; (ii) a temperature stability of 90% or more, relative to initial activity, at pH 7.5 determined after incubation for 20 minutes at 60°C in the absence of substrate; (iii) a temperature stability of 64% or more, relative to initial activity, at pH 7.5 determined after incubation for 20 minutes at 70°C in the absence of substrate; and (iv) an ability to hydrolyze a substrate containing Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr, or Val at its N-terminus.

Applicants assert that limiting the claims to SEQ ID NOs:1 and 2 would not adequately protect the inventors. Based on the teachings of the present application, one skilled in the art could find another aminopeptidase having the properties of the aminopeptidases of the present invention and thereby attempt to circumvent the literal scope of Applicants' patent rights. Thus, a competitor seeking to avoid infringing the claims would merely have to follow the disclosure in the subsequently-issued patent to find a substitute.

Applicants have provided a detailed written description on how to isolate and identify the aminopeptidases and the nucleic acid sequences encoding such aminopeptidases. Applicants detail on page 5, line 18, to page 6, line 17, of the specification, instructions for performing standard Southern hybridization under medium, medium-high, and high stringency conditions to identify such nucleic acids from other strains, whether of the same or different genera or species. In short, the hybridization methods describe the use of specific probes in written and enabling detail for identifying other aminopeptidase genes which

hybridize under medium, medium-high, or high stringency conditions with the probes. The probes described in the specification are nucleotides 46 to 1488 of SEQ ID NO:1, or its complementary strand. One of ordinary skill in the art would recognize that the use of such probes under medium, medium-high, or high stringency conditions allows the identification of genes encoding other aminopeptidases that are closely related or essentially identical to the gene contained in SEQ ID NO:1. For example, Applicants have provided details in Example 7 for probing a library of an *Aspergillus* strain where eleven colonies produced strong hybridization signals with the probe.

Applicants also detail on page 4, line 9, to page 5, line 17, of the specification, a description for determining the degree of identity between two amino acid sequences by the Clustal method (Higgins, 1989, *CABIOS* 5: 151-153) Percent identity is determined by a direct consecutive comparison of the amino acids of the polypeptide corresponding to the amino acids of a reference polypeptide, *i.e.*, the amino acid sequence of SEQ ID NO:2 of the claimed invention. It is well known in the art that a protein that has 90% identity on the amino acid level to a reference protein, e.g., SEQ ID NO:2, is closely related to the reference protein. One of ordinary skill in the art would recognize that a nucleic acid sequence encoding such a polypeptide will have essentially the same inherent properties as the claimed reference polypeptide of SEQ ID NO:2. As the percent identity between the two proteins increases to 90%, 95%, and 97%, the two polypeptides having aminopeptidase activity will have essentially the same inherent properties.

One of ordinary skill in the art would further recognize that amino acid changes of SEQ ID NO:2 of a minor nature could be made, naturally or recombinantly, that do not change the inherent properties of the polypeptide of SEQ ID NO:2. Such amino acid changes include, for example, conservative amino acid substitutions that do not significantly affect the folding and/or activity of the protein; and small deletions, typically of one to about 30 amino acids. Such conservative substitutions are, for example, within the group of basic amino acids (arginine, lysine and histidine), acidic amino acids (glutamic acid and aspartic acid), polar amino acids (glutamine and asparagine), hydrophobic amino acids (leucine, isoleucine and valine), aromatic amino acids (phenylalanine, tryptophan and tyrosine), and small amino acids (glycine, alanine, serine, threonine and methionine). Amino acid substitutions which do not generally alter the specific activity are known in the art and are described, for example, by H. Neurath and R.L. Hill, 1979, *In*, *The Proteins*, Academic Press, New York. The most commonly occurring exchanges are Ala/Ser, Val/Ile, Asp/Glu, Thr/Ser, Ala/Gly, Ala/Thr, Ser/Asn, Ala/Val, Ser/Gly, Tyr/Phe, Ala/Pro, Lys/Arg, Asp/Asn, Leu/Ile, Leu/Val, Ala/Glu, and Asp/Gly as well as these in reverse. It would not be surprising to one skilled in the art that a protein containing, for example, 100 amino acids could easily be modified by making 3, 5, 10, or more conservative substitutions without changing the

inherent functional properties of the protein. Consequently, one skilled in the art could find another aminopeptidase having the properties of the aminopeptidases of the present invention and thereby attempt to circumvent the literal scope of Applicants' patent rights.

In the instant case, claims limited to SEQ ID NOs:1 and 2 would not adequately protect the inventors. Based on the teachings of the present application, one skilled in the art could find another nucleic acid sequence encoding a polypeptide having essentially the same properties of the polypeptide of SEQ ID NO:2 and thereby attempt to circumvent the literal scope of Applicants' patent rights based on any of the circumstances described above.

Applicants submit that the information disclosed in the specification combined with the knowledge of the art provides sufficient guidance to one of ordinary skill in the art to isolate such aminopeptidases. The written description as a whole is sufficient to inform the skilled artisan that Applicants were in possession of the claimed aminopeptidases at the time the application was filed.

For the foregoing reasons, Applicants submit that the rejections under 35 U.S.C. § 112, first paragraph, have been overcome and respectfully request reconsideration and withdrawal of the rejections.

II. The Rejection of Claims 130-133, 136-145, 151-155, and 158-168 under 35 U.S.C. § 112, First Paragraph

Claims 130-133, 136-145, 151-155, and 158-168 stand rejected under 35 U.S.C. § 112, first paragraph, "as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention." Specifically, the Office Action states:

> The specifications guidance does not enable a skilled artisan to obtain the appropriate peptides. The specification does not provide a specific probe, hybridization conditions or a library from which an aminopeptidase with the claimed characteristics may be isolated. The artisan, based on the limited guidance is not reasonably assured of reproducibly and reliably obtaining the claimed aminopeptidases as directed. Further, the artisan would be forced, after obtaining a candidate agent to perform undue experimentation to determine the full open reading frame, express the protein and determine the peptides biological properties as well as its functional characteristics. The specification is required to be fully enabled at the time of the invention. However, applicants do not provide evidence of reduction to practice for any other aminopeptidase other than that of SEQ ID NO:2. The specification merely invites the artisan to discover other related sequences. The single species does not support the genus claim.

This rejection is respectfully traversed.

The Examiner suggests that the specification is insufficient to enable one skilled in the art to practice the invention as broadly claimed without undue experimentation.

Applicants respectfully disagree. Applicants submit that undue experimentation would not be required to practice the invention because Applicants' disclosure provides considerable direction and guidance on how to practice their invention and presents working examples. There was a high level of skill in the art at the time when the application was filed, and Applicants' enabling disclosure in combination with that skill in the art provides sufficient knowledge needed to practice the invention.

For the same reasons explained in section III (see above) and further reasons described below, Applicants assert that the specification enables any person skilled in the art to practice the invention commensurate in scope with the instant claims. Specifically, Applicants have provided detailed methods for isolating the claimed aminopeptidase and determining whether they fall within the scope of protection sought by Applicants using Applicants' enabling methods for preparing and probing DNA libraries (see Example 4-7); for isolating nucleic acids encoding the aminopeptidase (see Example 7); for determining cross-hybridization of the nucleic acids encoding the aminopeptidase using nucleotides 46 to 1488 of SEQ ID NO:1, or their complementary nucleotides (se page 5, line 18, to page 6, line 18); for comparing the percent identity of the deduced amino acid sequence of the aminopeptidase to amino acids 16 to 496 of SEQ ID NO:2 using the Clustal method according to Higgins, 1989, *CABIOS* 5: 151-153 (see page 4, line 9, to page 5, line 17); for expressing the nucleic acid sequence encoding an aminopeptidase in a host cell (see Examples 9-10 and 12-14); for purifying the aminopeptidase (see Example 11); and for characterizing the properties of the aminopeptidase (see Example 16). Applicants assert that it is well within the skill of the art to practice the invention using the Applicants' enabling disclosure without undue experimentation. The methods for isolating the claimed aminopeptidases and determining the properties of the claimed aminopeptidases are described in the specification in enabling detail for practicing the claimed invention in a predictable manner. On the basis of Applicants' disclosure, one skilled in the art would know how to identify and isolate such aminopeptidases. Applicants, therefore, submit that the information disclosed in the specification enables one skilled in the art to isolate the claimed aminopeptidases.

The Examiner also suggests that Applicants are not enabled for the use of a vector or host cell expressing the complementary strand or hybridizing sequences which encode an aminopeptidase because the protein encoded by the opposite strand is unrelated both structurally and functionally to the aminopeptidase sequences. Applicants respectfully disagree with the Examiner. A gene is comprised of two strands which are complementary to each other. A nucleic acid sequence is complementary to another if it is able to form a perfectly hydrogen-bonded duplex with it, according to the Watson-Crick rules for base pairing; and a mRNA molecule is complementary to one of the DNA strands of the gene that

encodes it. Thus, the complementary strand or hybridizing sequences could be used to clone a gene encoding an aminopeptidase because the complementary strand or hybridizing sequences are related both structurally and functionally to the aminopeptidase encoding sequences.

Applicants, therefore, assert that the specification is sufficiently enabling to practice the claimed invention.

For the foregoing reasons, Applicants submit that the rejections under 35 U.S.C. § 112, first paragraph, have been overcome and respectfully request reconsideration and withdrawal of the rejections.

III. The Rejection of Claims 146 and 169 under 35 U.S.C. § 112, First Paragraph

Claims 146 and 169 stand rejected under 35 U.S.C. § 112, first paragraph, "as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention." Specifically, the Office Action states that Applicant's statement under 37 CFR 1.808 under the Budapest Treaty and over the attorney's signature that all restrictions on the availability to the public of the deposited material, plasmid pEJG18 in *E. coli* NRRL B-21677, will be irrevocably removed upon the granting of the U.S. patent and that the deposit will be maintained for (a) thirty years, (b) at least five years after the most recent request for the furnishing of a sample of the deposit is received by the depository, or (c) the enforceable life of the U.S. patent granted from this application, whichever is longest, is an insufficient assurance that all required deposits have been made and all the conditions of 37 CFR 1.801-1.809 have been met. Specifically, the Office Action requests that amendment of the specification to recite the date of deposit and the complete name and full address of the depository is required.

This rejection is respectfully traversed.

Applicants respectfully point out that on page 54, line 27, to page 55, line 2, of the specification, the date of deposit and the complete name and full address of the depository are provided. Applicants therefore submit that the rejections have been overcome.

IV. The Rejection of Claims 143, 162, and 163 under 35 U.S.C. § 112, First Paragraph

Claims 143, 162, and 163 stand rejected under 35 U.S.C. § 112, first paragraph, "as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. The Office Action states::

> Claims 143, 162, and 163 are rejected as the claims recite medium

stringency conditions as hybridization under 35% formamide. The recitation of 50% formamide as medium stringency is considered new matter. The claim should be amended to either recite high striungency conditions, or alternatively 35% formaide which is consistent with the specification.

Applicants have cancelled claims 143, 162, and 163, but have corrected the typographical error in the new claims. The Examiner is correct in noting that claims should recite medium stringency conditions as hybridization under 35% formamide, and not under 50% formamide.

For the foregoing reason, Applicants submit that the rejections under 35 U.S.C. § 112, first paragraph, have been overcome and respectfully request reconsideration and withdrawal of the rejections.

V. The Rejection of Claims 130-133, 136-145, 151-155, and 158-168 under 35 U.S.C. § 102

Claims 130-133, 136-145, 151-155, and 158-168 stand rejected under 35 U.S.C. § 102(b) as being anticipated by Nishizawa *et al.* (J. Biol. Chem. 269:13651-13655, 1994). The Office Action states:

> Nishizawa disclose a *S. cerevisiae* aminopeptidase which hybridizes with SEQ ID NO:1 as the Nishizawa sequence encodes residues 255-264 of SEQ ID NO:2. This 30 mer has a Tm=87 degrees C based on the formula Tm=4(G+C) + 2(A+T). Therefore, the sequence hybridizes under medium and high stringency conditions at 42 degrees C. Thus, the sequence anticipates the claimed invention, see in particular residues 1180-1209 of the Nishizawa nucleic acid sequence.

This rejection is respectfully traversed.

Nishizawa *et al.* disclose a *Saccharomyces cerevisiae* aminopeptidase Y which is a vacuolar enzyme consisting of 537 amino acids.

However, Nishizawa *et al.* do not disclose the aminopeptidases claimed herein. The claimed aminopeptidases are not vacuolar enzymes. The Office Action indicates that a sequence comparison shows that the deduced amino acid sequence of the *Saccharomyces cerevisiae* aminopeptidase Y is 31.3% identical to the deduced amino acid sequence of the aminopeptidase of the instant invention. This low degree of identity between the amino acid sequences of the two aminopeptidases indicates that the corresponding genes, and subsequences of the genes which encode polypeptides having aminopeptidase activity, would not hybridize under medium stringency conditions as defined by prehybridization and hybridization at 42°C in 5X SSPE, 0.3% SDS, 200 μg/ml sheared and denatured salmon sperm DNA, and 35% formamide, following standard Southern blotting procedures.

For the foregoing reasons, Applicants submit that this rejection under 35 U.S.C. § 102 has been overcome. Applicants respectfully request reconsideration and withdrawal of the

rejection.

VI. The Rejection of Claims 130-169 under 35 U.S.C. § 102

Claims 130-169 stand rejected under 35 U.S.C. § 102(b) as being anticipated by Nakadai *et al.* (*Agr. Biol. Chem.* 37 (4): 767-774, 1973). The Office Action states:

> Nakadai teach a MW 61 kD polypeptide which shares the characteristics of applicants aminopeptidase, i.e., it is stable at 60 degrees, see in particular figure 9, has a pH optimum within applicants range, see in particular figures 6-8 and hydrolyzes oligopeptides at the amino terminus with particular affinity to leucine, but also cleaves other N-terminus amino acids, see in particular figure 1, 10, and Table 2. The molecular weight of the peptide is considered to be so close as to be equivalent to 58 kD. The peptide originates from Asp. oryzae. Nakadai thus appears to correlate to applicants peptide and inherently shares the amino acid sequence ...

This rejection is respectfully traversed.

Nakadai *et al.* disclose the purification and properties of leucine aminopeptidase II from *Aspergillus oryzae*. However, Nakadai *et al.* do not disclose the aminopeptidases claimed herein.

preamble

The Nakadai leucine aminopeptidase II is stable up to 60°C for 10 minutes, but loses its activity almost completely at 70°C for 10 minutes (see Figure 9 of the cited reference). On the other hand, the claimed aminopeptidases have a temperature stability of 64% or more, relative to initial activity, at pH 7.5 determined after incubation for 20 minutes at 70°C.

For the foregoing reasons, Applicants submit that this rejection under 35 U.S.C. § 102 has been overcome. Applicants respectfully request reconsideration and withdrawal of the rejection.

VII. The Rejection of Claims 130-169 under 35 U.S.C. § 102

Claims 130-169 stand rejected under 35 U.S.C. § 102(b) as being anticipated by Nakadai *et al.* (*Agr. Biol. Chem.* 37 (4): 775-782, 1973). The Office Action states:

> Nakadai teach a MW 56 kD polypeptide which shares the characteristics of applicants aminopeptidase, i.e., it is stable at 60 degrees, see in particular figures 8-9, has a pH optimum within applicants range, see in particular figures 6-7 and hydrolyzes oligopeptides at the amino terminus with particular affinity to leucine, but also cleaves other N-terminus amino acids, see in particular Table 2. The molecular weight of the peptide is considered to be so close as to be equivalent to 58 kD. The peptide originates from Asp. oryzae. Nakadai thus appears to correlate to applicants peptide and inherently shares the amino acid sequence ...

This rejection is respectfully traversed.

Nakadai *et al.* disclose the purification and properties of leucine aminopeptidase II from *Aspergillus oryzae*. However, Nakadai *et al.* do not disclose the aminopeptidases claimed herein.

The Nakadai leucine aminopeptidase III is stable up to 60°C for 10 minutes, but loses its activity almost completely at 70°C for 10 minutes (see Figure 10 of the cited reference). On the other hand, the claimed aminopeptidases have a temperature stability of 64% or more, relative to initial activity, at pH 7.5 determined after incubation for 20 minutes at 70°C.

For the foregoing reasons, Applicants submit that this rejection under 35 U.S.C. § 102 has been overcome. Applicants respectfully request reconsideration and withdrawal of the rejection.

VIII. Conclusion

In view of the above, it is respectfully submitted that all claims are in condition for allowance. Early action to that end is respectfully requested. The Examiner is hereby invited to contact the undersigned by telephone if there are any questions concerning this amendment or application.

Respectfully submitted,

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